Influence of supplemental dietary poultry fat on the yolk characteristics of commercial layers inoculated before or at the onset of lay with F-strain *Mycoplasma gallisepticum*[1,2]

S. W. Park,* M. R. Burnham,* S. L. Branton,† P. D. Gerard,‡ S. K. Womack,* and E. D. Peebles*[3]

**Department of Poultry Science, and †Poultry Research Unit, Agricultural Research Service, USDA, Mississippi State, MS 39762; and ‡Department of Applied Economics and Statistics, Clemson University, Clemson, SC 29634*

ABSTRACT Effects of F-strain *Mycoplasma gallisepticum* (FMG) inoculation and 1.5% supplemental dietary poultry fat (PF) on the egg yolk characteristics of commercial layers at 24, 34, 44, 50, and 58 wk of age were investigated. Sham and FMG inoculations were administered at 12 and 22 wk of age and dietary treatments (basal control and basal control with 1.5% supplemental PF) were initiated at 20 wk of age. Yolk lipid concentration was reduced on wk 24 in birds that had been inoculated at 12 or 22 wk of age with FMG. The use of 1.5% supplemental PF increased percentage of yolk weight and yolk:albumen ratio across age and inoculation treatment. At 58 wk of age, concentrations of yolk palmitic acid increased and those of oleic and linolenic acid decreased when sham inoculations were given at 22 rather than at 12 wk of age. However, FMG inoculations given at 22 rather than at 12 wk increased palmitoleic acid and decreased stearic acid yolk concentrations. At 12 wk of age, FMG inoculations decreased yolk palmitoleic, oleic, and linolenic acid concentrations while causing increased yolk stearic and arachidonic acid levels when compared with sham inoculations. Furthermore, 1.5% supplemental PF decreased concentrations of palmitic and oleic acid and increased those of linoleic acid in the yolk at 58 wk of age. Despite the interaction of 1.5% supplemental PF with the prelay inoculation of FMG on early (18 to 26 wk) layer performance noted in a previous report, the effects of a prelay FMG inoculation and 1.5% supplemental PF on the egg yolk characteristics examined in the current study were independent of each other. This suggests that 1.5% supplemental PF is not effective in modulating the effects of an FMG inoculation at 12 wk of age on hen egg yolk characteristics between 24 and 58 wk of age and that the combined effects of PF supplementation and FMG inoculation on performance do not influence egg yolk characteristics.

Key words: egg yolk, F-strain *Mycoplasma gallisepticum*, inoculation, layer, poultry fat

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INTRODUCTION

Mycoplasma gallisepticum (**MG**) has been cultured from the liver of chickens (Sahu and Olson, 1976), may remain viable for 18 wk in egg yolk at 37°C (Chandiramani et al., 1966), and its transmission through the hatching egg is the major route of infection for the next generation (Kleven, 1981; Glisson and Kleven, 1984). According to Burnham et al. (2003), F-strain MG (**FMG**) infection affects the total yolk lipid (**YL**), cholesterol (**YCHOL**), and fatty acid concentrations in eggs of laying hens. Compared with control birds, Burnham et al. (2003) found that YL was higher at 32 and 44 wk and lower at 22 and 48 wk, and that YCHOL was lower at 28 wk subsequent to an FMG inoculation at 12 wk. Also, concentrations of yolk linoleic, stearic, and arachidonic acids were increased, whereas concentrations of myristic, palmitoleic, and oleic acids were decreased in FMG-treated birds compared with controls. It was suggested that FMG colonization in the liver of laying hens may affect egg production through alterations in YL and the metabolism and production of various fatty acids that are ultimately deposited in the yolk.

Peebles et al. (2003) determined the effects of the use of 1.5% supplemental dietary poultry fat (**PF**) beginning at 20 wk of age on layer performance through 26 wk of age after an inoculation with FMG at 12 wk of age. Reduced feed consumption as a result of feeding the supplemental PF in sham-inoculated birds was

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[3]Corresponding author: dpeebles@poultry.msstate.edu

ameliorated when supplementation was preceded by an FMG inoculation. The supplemental PF was also found to alleviate reductions in early egg production due to the prelay FMG infection. Furthermore, in a previous companion article to the current study, Park et al. (2009) reported that 1.5% supplemental PF reduced feed consumption across a 20 to 55-wk laying cycle in layers that had likewise experienced a wk 12 inoculation (sham or FMG). The objective of this study was, therefore, to examine the possible effects of 1.5% supplemental PF, subsequent to an FMG inoculation at 12 wk of age, on the yolk characteristics of commercial egg-laying hens throughout lay.

MATERIALS AND METHODS

In all 3 trials, 1-d-old Hy-Line W-36 Single Comb White Leghorn pullets were obtained from a commercial hatchery certified free of MG and *Mycoplasma synoviae* (USDA-APHIS-VS, 2003). Until 12 wk of age, birds were raised, fed, vaccinated, and tested for the presence of MG and *M. synoviae* as described by Peebles et al. (2003). At 12 and 22 wk of age in each trial, 120 sham (control)- and 120 FMG (treated)-inoculated birds were wing-banded and randomly assigned to individual cages in 1 of 2 enclosed and isolated ends of a caged layer facility according to inoculation treatment. Sham and FMG inoculations at 12 and 22 wk and *Mycoplasma* detection during lay were as described by Peebles et al. (2003) and Park et al. (2009).

Two isocaloric and isonitrogenous treatment layer diets were randomly provided to birds within each end of the layer house at 20 wk of age, with both dietary treatments assigned to birds belonging to each inoculation type (sham- or FMG-inoculated) and inoculation age (12 or 22 wk) treatment combination. One diet served as a basal control diet (**BC**; contained 0.5% PF), and the other was the basal diet supplemented with 1.5% PF (**BCPF**; 2.0% total PF). There were 3 replicate groups (10 birds per replicate group) for each inoculation type, inoculation age, and dietary treatment combination. Feed and water were provided for ad libitum consumption, and birds in each side of the house were watered, fed, and ventilated separately. Descriptions of the diets fed to the layers are provided by Peebles et al. (2003) and Park et al. (2009). All pullet and layer diets were formulated to meet or exceed NRC (1994) specifications, and all trials were conducted under an approved USDA animal care and use protocol.

At 24, 34, 44, 50, and 58 wk of age, a total of 10 eggs were collected from each replicate unit for internal egg quality determinations. If less than 10 eggs were collected on a given day, the rest were collected the following day of the same week. Nevertheless, determinations were made on the same day that eggs were collected. Internal egg quality parameters that were determined at all 5 ages and in all 3 trials included percentage of yolk weight (**PYW**), percentage of albumen weight (**PAW**), yolk:albumen ratio (**YAR**), and concentrations of yolk moisture (**YM**) and YL. Yolk cholesterol concentrations were determined at wk 24 and 58 in 2 of the 3 trials, and yolk fatty acid profiles were determined at wk 58 in all 3 trials. After eggs were weighed, they were broken out for subsequent internal egg quality determinations. Yolk and albumen weight percentages were each based on total fresh egg weight. Yolk:albumen ratio was expressed as a fraction of the PYW (numerator) and PAW (denominator). Quantitation of YM and YL were as described by Burnham et al. (2003). Yolk moisture and lipid concentrations were expressed as percentages of fresh yolk sample weight. Determination of YCHOL was performed by direct saponification followed by a procedure that utilized capillary liquid gas chromatography. Methyl esterification of YL was as described by Burnham et al. (2003). Methylated samples were sealed and were stored at −20°C until subsequent fatty acid analysis by gas chromatography. All chromatographic analyses were performed according to procedures of the Association of Official Analytical Chemists (1980). The individual fatty acids retained by the gas chromatograph were expressed as a percentage of total methyl esters of the fresh yolk sample, and YCHOL was expressed in milligrams per gram of total fresh yolk sample.

Statistical Analysis

Average total egg, yolk, and albumen weights within each replicate were calculated by dividing total replicate weight by the total number of samples weighed. Yolk samples within each replicate group for YM, YL, YCHOL, and fatty acid analyses were pooled before content analysis, and all other individual sample data within each replicate group were averaged before data analysis. A completely randomized experimental design, with trial as a block, was utilized. The data of all 3 trials were pooled and then analyzed together. Therefore, the results from each trial were not reported independently but were reported over all 3 trials. Trial was considered as a random effect. Parameters for which the same experimental units were observed over multiple age periods were subjected to a repeated measures analysis. These included all parameters examined except yolk fatty acids. Yolk fatty acid concentrations at wk 58 were subjected to split-plot analysis to assess the effects of diet, inoculation type, and inoculation age. Comparisons of means were by Fisher's (protected) least significant difference method in the event of significant global effects (Steel and Torrie, 1980; Petersen, 1985; Freund and Wilson, 1997), and all data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003). Global effects and means separations were considered significant at $P \leq 0.05$.

RESULTS

There were significant ($P \leq 0.0001$) hen age main effects for PYW, PAW, YAR, and YM between 24 and

Table 1. Relative egg yolk [PYW; (g of yolk/g of egg) × 100] and albumen [PAW; (g of albumen/g of egg) × 100] weights, yolk:albumen ratio (YAR), and yolk moisture (YM) and cholesterol (YCHOL) concentrations at 24, 34, 44, 50, and 58 wk of hen age across inoculation type, inoculation age, and dietary treatment[1]

Weeks of age	PYW (%)	PAW (%)	YAR	YM (%)	YCHOL (mg/g)
24	24.6^{d}	62.8^{a}	0.392^{d}	49.8^{c}	12.1
34	27.8^{c}	60.5^{b}	0.460^{c}	50.5^{b}	—
44	29.2^{b}	59.2^{c}	0.494^{b}	52.5^{a}	—
50	29.6^{a}	58.7^{d}	0.505^{a}	50.3^{bc}	—
58	29.7^{a}	58.7^{d}	0.507^{a}	50.9^{b}	12.1
Pooled SEM	0.13	0.45	0.0050	1.36	0.48

[a–d]Means among week of age within a column (parameter) with no common superscript differ ($P \leq 0.05$).

[1]n = 72 (2 diets × 2 inoculation types × 2 inoculation ages × 3 repetitions × 3 trials) replicate units used for the calculation of the mean of each parameter at each week.

58 wk of age. Means for each of these parameters at 24, 34, 44, 50, and 58 wk of hen age across inoculation type, inoculation age, and dietary treatment are provided in Table 1. Percentage of yolk weight and YAR increased between wk 24 and 34, 34 and 44, and 44 and 50, whereas means at wk 50 and 58 were not different. Oppositely, PAW decreased between wk 24 and 34, 34 and 44, and 44 and 50, whereas means at wk 50 and 58 were not different. On the other hand, YM was highest at wk 44, and YM at wk 34 and 58 were higher than that at wk 24, with wk 50 intermediate. There were no significant effects of any kind noted for YCHOL; however, YCHOL means at 24 and 58 wk are included in Table 1 for added reference. There was a significant ($P \leq 0.0008$) hen age × inoculation type interaction for YL between 24 and 58 wk of age. The YL means for each type of inoculation treatment at 24, 34, 44, 50, and 58 wk of hen age across inoculation age and dietary treatment are provided in Table 2. At 24 wk of age, YL was reduced in birds that were FMG-inoculated (12 or 22 wk of age) in comparison to those that were sham-inoculated. Also, between 24 and 58 wk of age, there were significant ($P \leq 0.05$) diet main effects for PYW and YAR. The PYW and YAR means for each dietary treatment across sampling age period, inoculation type, and inoculation age are provided in Table 3. The addition of 1.5% supplemental PF to the basal diets of the birds increased PYW and YAR.

Inoculation type × inoculation age interactions were found for yolk myristic ($P \leq 0.03$), palmitic ($P \leq 0.02$), palmitoleic ($P \leq 0.03$), stearic ($P \leq 0.004$), oleic ($P \leq 0.002$), linolenic ($P \leq 0.005$), and arachidonic ($P \leq 0.008$) acid concentrations at 58 wk of age. The means of each of these parameters for each type and age of inoculation across dietary treatment are provided in Table 4. Compared with those in birds that were sham-inoculated at 12 wk of age, yolk concentrations of palmitic acid in wk 22 sham-inoculated birds and arachidonic acid in wk 12 FMG-inoculated birds were higher, whereas yolk oleic and linolenic acid concentrations were lower in wk 22 sham-inoculated and wk 12 FMG-inoculated birds. In addition, compared with those in birds that were inoculated with FMG at 22 wk or that were sham-inoculated at 12 wk, yolk palmitoleic acid levels were lower and yolk stearic acid levels were higher in birds that were inoculated with FMG at 12 wk of age. Furthermore, there was a significant diet main effect for concentrations of yolk palmitic ($P \leq 0.0001$), oleic ($P \leq 0.005$), and linoleic acid ($P \leq 0.0001$) at 58 wk of age. The means of each of these parameters for each dietary treatment across inoculation type and inoculation age are provided in Table 5. Yolk concentrations of palmitic and oleic acids were lower, whereas those of linoleic acid were higher, in birds fed the BCPF diet in comparison to the BC diet.

DISCUSSION

A delay in onset of lay by 1 wk and a decrease in total egg production (Burnham et al., 2002b), as well as significant reductions in the average number of mature ovarian follicles and alterations in liver, ovarian, and oviduct characteristics (Burnham et al., 2002a), have been reported to occur in commercial layers in response to the inoculation of FMG at 12 wk of age. In a later report, Burnham et al. (2003) concluded that the effects of FMG on egg production are associated with alterations in YL and the metabolism and production of various fatty acids that are deposited in the yolk subsequent to the colonization of FMG in the livers of laying hens. *Mycoplasma gallisepticum* has the ability to invade cells (Winner et al., 2000), and Sahu and

Table 2. Yolk lipid concentration for sham- and F-strain *Mycoplasma gallisepticum* (FMG)-inoculated birds at 24, 34, 44, 50, and 58 wk of age across inoculation age and dietary treatment[1,2]

Weeks of age	Sham (%)	FMG (%)
24	33.2^{a}	29.5^{b}
34	31.3	29.5
44	32.0	32.2
50	29.7	29.4
58	28.8	29.3

[a,b]Means among treatments within a row (week of age) with no common superscript differ ($P \leq 0.05$).

[1]n = 36 (2 diets × 2 inoculation ages × 3 repetitions × 3 trials) replicate units used for the calculation of the mean of each treatment at each week.

[2]Pooled SEM = 1.35.

Table 3. Relative egg yolk weight [PYW; (g of yolk/g of egg) × 100] and yolk:albumen ratio (YAR) in birds fed basal control diets (BC) and basal control diets supplemented with 1.5% poultry fat (BCPF) across sampling age period, inoculation type, and inoculation age[1]

Parameter	BC	BCPF	Pooled SEM
PYW (%)	28.0[b]	28.3[a]	0.10
YAR	0.468[b]	0.474[a]	0.004

[a,b]Means among treatments within a row (parameter) with no common superscript differ ($P \leq 0.05$).

[1]n = 180 (5 age periods × 2 inoculation types × 2 inoculation ages × 3 repetitions × 3 trials) replicate units used for the calculation of treatment means for each parameter.

Olson (1976) noted that MG may be cultured from the livers of infected chickens. These findings suggest that MG may be capable of interfering with liver lipid metabolism. Liver colonization by FMG may, therefore, be a possible means by which egg production, liver lipid metabolism, and the subsequent lipid profiles of egg yolks in FMG-infected hens may be altered.

The results of this study indicate that the inoculation of layers with FMG at either 12 or 22 wk of age may subsequently decrease YL. However, between 24 and 58 wk of age, a significant decrease in YL only occurred initially at 24 wk of age. The absence of a significant effect at any of the succeeding time periods through wk 58 suggests that FMG affects YL for only a limited amount of time after inoculation. In a report by Burnham et al. (2003), which examined egg yolk composition between 22 and 58 wk of age, it was likewise shown that YL was consistently reduced in 2 consecutive trials only at 22 wk of age in layers that were inoculated with FMG at 12 wk of age. Razin and Tully (1970) and Edward (1971) reported that members of *Mycoplasma* genera require a substantial amount of exogenous cholesterol or its esters for growth and for incorporation into their membranes. Nevertheless, the hens in the present study were apparently able to eventually adapt physiologically to the nutritional demands of the FMG, so that YL was not affected by wk 34 or after 22 wk of infection.

Burnham et al. (2002b) examined the potential effects of an FMG inoculation at 12 wk of age on the egg characteristics of commercial layers and noted that it did not affect PYW. Similarly, in the current study, FMG inoculation had no influence on PYW. However, the BCPF diet significantly increased PYW and YAR over that of the BC diet across wk 24, 34, 44, 50, and 58. Although Sell et al. (1987) observed after 4 to 12 wk of dietary treatment that the absolute yolk weights of eggs from White Leghorn hens increased linearly with increases in the level (3.0 or 6.0%) of animal-vegetable fat added to diets, PYW did not increase significantly due to associated increases in egg weight. As in the current study, the supplemented diets used by Sell et al. (1987) were isocaloric and isonitrogenous to the basal diet while the level of total fat in the diet was increased. The increase in PYW with dietary PF supplementation in the present report would be related to the lack of an associated increase in egg weight, as noted in the earlier companion report by Park et al. (2009). Conversely, upon examination of the effects of PF level (1.5 or 3.0%) apart from the associated influences of energy level in the diets of broiler breeder hens, Peebles et al. (2000) did not observe a significant effect on PYW, YAR, or egg weight.

Changes in the yolk concentrations of linoleic acid between 24 and 44 wk of age and of myristic, stearic, palmitoleic, oleic, and arachidonic acid between 24 and 58 wk of age have been found to occur in layers in response to the inoculation of FMG at 12 wk of age (Burnham et al., 2003). More specifically, decreases in myristic, palmitoleic, and oleic acid and increases in linoleic, stearic, and arachidonic acid occurred after the inoculation of FMG at 12 wk. Similarly, in comparison to sham inoculations at 12 wk in this study, decreases in palmitoleic and oleic acids and increases in stearic and arachidonic acids were also found in the yolks of eggs from hens at 58 wk of age in response to FMG inoculations at 12 wk. The results of the current study confirm those of Burnham et al. (2003), that an FMG inoculation at 12 wk in layers subsequently reduces yolk concentrations of palmitoleic and oleic acid and increas-

Table 4. Yolk myristic, palmitic, palmitoleic, stearic, oleic, linolenic, and arachidonic acid concentrations (% of methyl esters) at 58 wk of age within type of inoculation [sham and F-strain *Mycoplasma gallisepticum* (FMG)] and age of inoculation (12 and 22 wk) across dietary treatment[1]

	Sham (%)		FMG (%)		
Fatty acid	12 wk	22 wk	12 wk	22 wk	Pooled SEM
Myristic acid	0.254	0.244	0.240	0.255	0.0289
Palmitic acid	28.2[b]	28.9[a]	28.6[ab]	28.7[ab]	1.10
Palmitoleic acid	1.75[a]	1.67[ab]	1.56[b]	1.70[a]	0.154
Stearic acid	12.9[b]	13.3[ab]	13.7[a]	13.2[b]	0.90
Oleic acid	33.8[a]	32.6[b]	32.5[b]	33.3[ab]	1.93
Linolenic acid	0.283[a]	0.254[b]	0.240[b]	0.261[ab]	0.0306
Arachidonic acid	5.23[b]	5.48[ab]	5.79[a]	5.42[ab]	0.729

[a,b]Means among treatments within a row (fatty acid) with no common superscript differ ($P \leq 0.05$).

[1]n = 18 (2 diets × 3 repetitions × 3 trials) replicate units used for the calculation of treatment means for each parameter.

Table 5. Concentrations (% of methyl esters) of yolk palmitic acid, oleic acid, linoleic acid, and all other remaining fatty acids combined (other; not including myristic, palmitoleic, stearic, linolenic, and arachidonic acids) at 58 wk of age in birds fed basal control diets (BC) and basal control diets supplemented with 1.5% poultry fat (BCPF) across inoculation type and inoculation age[1]

Fatty acid	BC (%)	BCPF (%)	Pooled SEM
Palmitic acid	28.9[a]	28.3[b]	1.08
Oleic acid	33.5[a]	32.6[b]	1.88
Linoleic acid	12.8[b]	13.7[a]	1.04
Other	3.93[b]	4.42[a]	1.580

[a,b]Means among dietary treatment within a row (fatty acid) with no common superscript differ ($P \leq 0.05$).

[1]n = 36 (2 inoculation types × 2 inoculation ages × 3 repetitions × 3 trials) replicate units used for the calculation of the treatment means for each parameter.

es those of stearic and arachidonic acid. Conversely, myristic and linoleic acids were not affected by inoculation treatment, whereas linolenic acid was lower in 12 wk FMG compared with 12 wk sham treatment birds in the present study. These associated yolk fatty acid changes may have nevertheless occurred through the preferential utilization of various fatty acids by FMG and subsequent coordinated shifts in the activities of various liver lipid enzymes as described by Burnham et al. (2003). In addition, palmitoleic acid was lower and stearic acid was higher in yolks from birds inoculated with FMG at 12 rather than at 22 wk in this experiment. These data would further suggest that the age at which FMG is inoculated (12 or 22 wk) may affect yolk palmitoleic and stearic acid levels, such that when FMG is administered at 22 rather than at 12 wk of age, its effects on the yolk levels of these 2 fatty acids are lost when compared with sham-inoculated controls.

The results of the current report also show that diet exerts effects on several yolk fatty acids that are independent of those in response to inoculation treatment, and that changes in the concentrations of these fatty acids in the yolk do not reflect their respective changes in the diet after the addition of 1.5% supplemental PF. Peebles et al. (2003) previously reported the fatty acid contents of the diets used in the current study and noted that in comparison to the BC diet, palmitic and oleic acid concentrations were higher and concentrations of linoleic acid were lower in the BCPF diet. However, in the current report, the yolk concentrations of palmitic and oleic acid were lower and those of linoleic acid were higher in eggs from hens that were fed the BCPF diet in comparison to those fed the BC diet. Therefore, changes in the yolk concentrations of these 3 fatty acids were opposite to those in the diet after the addition of 1.5% supplemental PF.

In conclusion, the inoculation of layers with FMG (12 or 22 wk) may decrease YL early in lay, and the inoculation of FMG specifically at 12 wk can alter the fatty acid profile of egg yolks toward the end of lay. These effects are independent of those exerted by the addition of 1.5% supplemental PF, which may increase PYW and YAR throughout lay. Furthermore, the effects of the inoculation and dietary treatments on these yolk characteristics were independent of those on egg production, as previously reported for these same birds by Park et al. (2009).

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